BOX 8-1

Methods for Studying T Cell Activation

Our current knowledge of the cellular events in T cell activation is based on a variety of experimental techniques in which different populations of T cells are activated by defined stimuli and functional responses are measured. *In vitro* experiments have provided a great deal of information on the changes that occur in a T cell when it is stimulated by antigen. More recently, several techniques have been developed to study T cell proliferation, cytokine expression, and anatomic redistribution in response to antigen activation *in vivo*. The new experimental approaches have been particularly useful for the study of naive T cell activation and the localization of antigen-specific memory T cells after an immune response has waned.

POLYCLONAL ACTIVATION OF T CELLS ***Polyclonal activators*** of T cells bind to many or all TCR complexes regardless of specificity and activate the T cells in ways similar to peptide-MHC complexes on APCs. Polyclonal activators are mostly used *in vitro* to activate T cells isolated from human blood or the lymphoid tissues of experimental animals. Polyclonal activators can also be used to activate T cells with unknown antigen specificities, and they can evoke a detectable response from mixed populations of naive T cells, even though the frequency of cells specific for any one antigen would be too low to elicit a detectable response. The polymeric plant proteins called lectins, such as concanavalin-A (Con-A) and phytohemagglutinin (PHA), are one commonly used group of polyclonal T cell activator. Lectins bind specifically to certain sugar residues on T cell surface glycoproteins, including the TCR and CD3 proteins, and thereby stimulate the T cells. Antibodies specific for invariant framework epitopes on TCR or CD3 proteins also function as polyclonal activators of T cells. Often, these antibodies need to be immobilized on solid surfaces or beads or cross-linked with secondary anti-antibodies to induce optimal activation responses. Because soluble polyclonal activators do not provide costimulatory signals that are normally provided by APCs, they are often used together with stimulatory antibodies to receptors for costimulators, such as anti-CD28 or anti-CD2. Superantigens, another kind of polyclonal stimulus, bind to and activate all T cells that express particular types of TCR β chain (see Chapter 15, Box 15–1). T cells of any antigen specificity can also be stimulated with pharmacologic reagents, such as the combination of the phorbol ester PMA and the calcium ionophore ionomycin, that mimic signals generated by the TCR complex.

ANTIGEN-INDUCED ACTIVATION OF T CELLS ***Polyclonal populations*** of normal T cells that are enriched for T cells specific for a particular antigen can be derived from the blood and peripheral lymphoid organs of individuals after immunization with the antigen. The immunization serves to expand the number of antigen-specific T cells, which can then be restimulated *in vitro* by adding antigen and MHC-matched APCs to the T cells. This approach can be used to study antigen-induced activation of a mixed population of previously activated ("primed") T cells expressing many different TCRs, but the method does not permit analysis of responses of naive T cells.

Monoclonal populations of T cells, which express identical TCRs, have been useful for functional, biochemical, and molecular analyses. The limitation of these monoclonal populations is that they are maintained as long-term tissue culture lines and therefore may have phenotypically diverged from normal T cells *in vivo*. One type of monoclonal T cell population that is frequently used in experimental immunology is an antigen-specific T cell clone. Such clones are derived by isolating T cells from immunized individuals, as described for polyclonal T cells, followed by repetitive *in vitro* stimulation with the immunizing antigen plus MHC-matched APCs and cloning of single antigen-responsive cells in semisolid media or in liquid media by limiting dilution. Antigen-specific responses can easily be measured in these populations because all the cells in a cloned cell line have the same receptors and have been selected for growth in response to a known antigen-MHC complex. Both helper and CTL clones have been established from mice and humans. Other monoclonal T cell populations used in the study of T cell activation include antigen-specific T cell hybridomas, which are produced like B cell hybridomas (see Box 3–1, Chapter 3), and tumor lines derived from T cells have been established *in vitro* after removal of malignant T cells from animals or humans with T cell leukemias or lymphomas. Although some tumor-derived lines express functional TCR complexes, their antigen specificities are not known, and the cells are usually stimulated with polyclonal activators for experimental purposes. The Jurkat line, derived from a human T cell leukemia cell, is an example of a tumor line that is widely used as a model to study T cell signal transduction.

TCR transgenic mice are a source of homogeneous, phenotypically normal T cells with identical antigen specificities that are widely used for *in vitro* and *in vivo* experimental analyses. If the rearranged α and β chain genes of a single TCR of known specificity are expressed as a transgene in mice, a majority of the mature T cells in the mice will express that TCR. If the TCR transgene is crossed onto a RAG-1– or RAG-2–deficient background, no endogenous TCR gene expression occurs and 100% of the T cells will express only the transgenic TCR. TCR transgenic T cells can be activated *in vitro* or *in vivo* with a single peptide antigen, and they can be identified by antibodies specific for the transgenic TCR. One of the unique advantages of TCR transgenic mice is that they permit the isolation of sufficient numbers of naive T cells of defined specificity to allow one to study functional responses to the first exposure to antigen. This advantage has allowed investigators to study the *in vitro* conditions under which antigen activation of naive T cells leads to differentiation into functional subsets such as T_H1 and T_H2 cells (see Chapter 12). Naive T cells from TCR transgenic mice can also be injected into normal syngeneic recipient mice, where they home to lymphoid tissues. The recipient mouse is then exposed to the antigen for which the transgenic TCR is specific. By use of antibodies that label the TCR transgenic T cells, it is possible to follow their expansion and differentiation *in vivo* and to isolate them for analyzing recall (secondary) responses to antigen *ex vivo*.

METHODS TO ENUMERATE AND STUDY FUNCTIONAL RESPONSES OF ANTIGEN-SPECIFIC T CELLS *IN VIVO* ***Fluorescent dyes*** can be used to study prolifer-

Continued on following page